Ohio Valley Society of Toxicology
Annual Meeting Program

November 6th, 2020

Virtual

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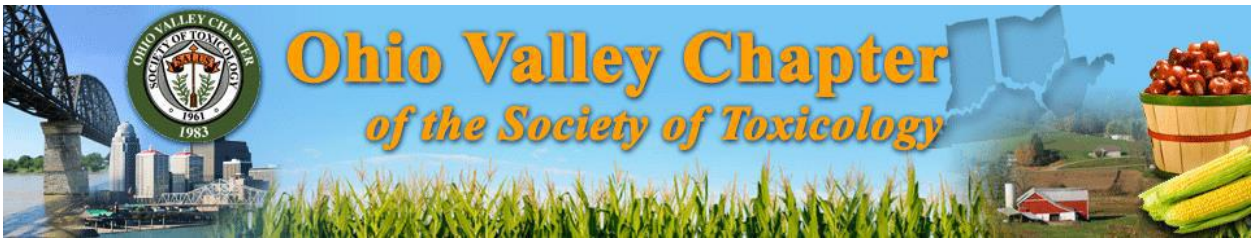
TABLE OF CONTENTS



OVSOT Annual Meeting Agenda
Friday, November 6, 2020

8:30 – 8:55 am	Entry into Meeting *(Moderator)*
9:00 - 9:15 am	Welcoming Remarks *(David Mattie)*
9:15 - 9:20 am	Introduction and Welcome *(Christopher Wingard)* Mr. John Launius, Greater Louisville Inc.
9:20 - 9:30 am	Meeting Logistics (acknowledge sponsors) Lu Cai, Ph.D., OVSOT President, 2020-2021
9:30 - 10:45 am	Doctoral Student Platform Presentations and Judging *(Jonathan Shannahan)*
10:45 - 12:00 pm	Poster viewing and judging *(Ana Cardoso/Jamie Young)*
12:00 - 1:00 pm	Lunch break; discussion with experts in government, academia and industry LiveinLou, Ms. Christine Tarquinio and Mr. Bradley Bringardner *(Christopher Wingard)*
1:00 - 2:00 pm	Tox-on-the-Clock *(Eddie Sloter)*
2:00 - 3:00 pm	Post-Doctoral Platform Presentations and Judging *(Jonathan Shannahan)*
3:00 - 3:15 pm	Break
3:15 - 4:15 pm	**Keynote: "Gerontogenic" Polypharmacy** *(David Mattie)*

Dr. Demetra Antimisiaris, PharmD, BCGP, FASCP
Associate Professor, Dept. Health Management & Systems Sciences
Director, Frazier Polypharmacy & Medication Management Program
Assistant Dean, Continuing Medical Education & Professional Development
University of Louisville, Louisville, KY

4:15 - 4:45 pm Announcement of Awards and Closing Remarks *(Jonathan Shannahan/Lu Cai)*

Keynote Speaker

Dr. Demetra Antimisiaris, PharmD, BCGP, FASCP
Associate Professor, Dept. Health Management & Systems Sciences
Director, Frazier Polypharmacy & Medication Management Program
Assistant Dean, Continuing Medical Education & Professional Development
University of Louisville, Louisville, KY

Bio:

Demetra (Dee) Antimisiaris is an Associate Professor, who joined the University of Louisville School of Public Health and Information Science in August of 2019. Dr. Antimisiaris started at the University of Louisville (UofL) in 2007 from the private sector, to lead the Polypharmacy Initiative which became the UofL Frazier Polypharmacy and Medication Management Program in 2016. The Frazier Polypharmacy Program is dedicated to education, research and public awareness regarding polypharmacy.

Dr. Antimisiaris was educated at the University of California at Davis, and the University of the Pacific where she graduated with a degree in clinical pharmacy (Doctor of Pharmacy or PharmD). Then, she completed a clinical pharmacy residency in Geriatric Pharmacotherapy at the VA Sepulveda (Los Angeles), a UCLA affiliated, interdisciplinary Geriatric Training Program (1989). She worked as a Consultant Pharmacist after graduating from residency where she reviewed

nursing home (long term care) charts for medication use optimization. Dr. Antimisiaris attributes her ability to assess medication use appropriateness to lessons learned from the dedicated staff who care for older persons in long term care as well as the VA-UCLA training program.

Dr. Antimisiaris believes “you can’t study what you don’t do”, so she makes a point to provide clinical consultation and participate in patient care while working to help grow the body of scholarship regarding polypharmacy. She has authored book chapters, served as guest editor of a Clinics in Primary Care Office Practice-Geriatrics edition, authored more than 50 journal pieces, and develops education sessions (a mission of the Frazier Polypharmacy Program). In 2019, she was awarded the American Society of Consultant Pharmacists Archambault Award. The Archambault Award is the Society’s highest honor conferred to an individual for their outstanding contributions to the profession.

Abstract: Polypharmacy (the use of multiple prescription, over the counter, supplements, and herbal products) has increased 85% in the past two decades. Exposure to polypharmacy must be considered as part of any research relative to aging. Typically, pharmacologic research is focused on target molecules, outcomes, and efficacy, to the exclusion of comprehensive toxicology, including longitudinal medication exposure. The baby boomers are the first generation of older adults who grew up and lived during the era of modern pharmaceutical manufacturing.

This seminar will examine hidden medication harms, even when medications are used appropriately and with good intent. We will also discuss molecular, pathophysiologic, and longitudinal polypharmacy exposure.

Objectives:

1. Differentiate a geriatric syndrome from a common disease of aging.

2. Identify a drug induced a pathophysiologic condition often misdiagnosed as organic disease.

3. Describe an example of hidden medication impact on pharmacodynamics or pharmacokinetics.

Doctoral Graduate Student Platform Presentations

9:30 - 10:45 am

PRESENTATION TITLES, NAME & ABSTRACTS

Toxicity of a novel lectin-Fc fusion protein in a human liver chimeric mouse model of hepatitis C infection

Matthew Dent, Nobuyuki Matoba

Department of Pharmacology and Toxicology, University of Louisville

Background: Hepatitis C virus (HCV) is an enveloped virus that can cause severe chronic liver disease, hepatocellular carcinoma, and ultimately death. Its densely glycosylated envelope protein is characterized by the high proportion of immature N-linked high-mannose glycans, which are not typically found in such levels on the surface of healthy normal cells. Thus, the presence of the high-mannose glycan cluster may be a useful biomarker or druggable target for HCV, though no such drug exists today that can utilize the unique carbohydrate biomarker. Our lab has developed a translational fusion protein consisting of the oligomannose-binding actinomycete-derived lectin Avaren and the Fc region of human IgG1 (AvFc), which showed cross-genotype neutralizing activity against HCV in vitro at nanomolar concentrations. Systemic administration of AvFc was well-tolerated in mice, rats, and rhesus macaques.

Objective: In this study we investigated the in vivo anti-HCV efficacy and safety of AvFc in a human liver chimeric mouse model.

Results: Systemic administration of 25 mg/kg AvFc every other day (Q2D) for 14 or 20 days (8 or 11 doses total, respectively), started on the day of a genotype 1a virus challenge, completely protected the human liver graft from infection, as demonstrated by RT-PCR analysis of blood HCV RNA levels. Additionally, a 25 mg/kg dose of AvFc Q2D for 20 days (11 total doses) did not result in significant toxicity to the animals. No significant fluctuations in body weight, human albumin, or human ALT levels were observed over the course of the study and an analysis of liver histopathology concluded that no

Conclusion: The safety and efficacy shown in this model provide crucial proof-of-concept evidence that AvFc may be an effective anti-HCV drug for use in the peri-operative setting to prevent reinfection of donor livers. One concern with any biologic, particularly that of xenogeneic origin like Avaren, is the potential for immunogenicity. A future study will assess the effects of prior administration of AvFc on its later efficacy.

Whale Cells Are Resistant to Cr(VI)-Induced Loss of Homologous Recombination Repair

Haiyan Lu, Sandra S. Wise, Jennifer H. Toyoda, Rachel M. Speer, Alicia Bolt, John Pierce, Wise, Sr.

Wise Laboratory of Environmental and Genetic Toxicology, Department of Pharmacology and Toxicology, University of Louisville, Louisville, Kentucky 40292

Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen, but the mechanism for Cr(VI)-induced cancer is uncertain. Chromosome instability (CIN) is a hallmark of lung cancer and is considered a major factor in Cr(VI)-induced lung cancer. Structural CIN can result from unrepaired DNA double strand breaks. Homologous recombination (HR) repair protects against these breaks. In human lung cells, we found Cr(VI) induces DNA double strand breaks while simultaneously inhibiting DNA double strand break repair, resulting in CIN. Whales face long-term exposure to Cr(VI) and accumulate Cr in their tissues, but appear to have a low incidence of cancer. Thus, to further explore the mechanism of Cr(VI)-induced lung cancer, we tested the hypothesis that whales are resistant to Cr(VI)-induced CIN. We measured the ability of Cr(VI) to induce DNA double strand breaks, HR repair, and chromosome damage in whale lung cells. We discovered Cr(VI) induces DNA strand breaks in whale cells, but whale cells avoid repair inhibition and maintain their HR repair response. Consequently, the amount of chromosomal damage was greatly reduced with no apparent CIN. By contrast, rats are a common model to study Cr(VI) though no studies have considered HR, DNA double strand breaks or HR repair. To determine if rats respond similarly to humans or more like whales, we compare DNA double-strand breaks and HR repair in rat lung cells and confirmed that Cr(VI)-induced genetic instability in the rat is similar to humans. Thus, future studies will determine the underlying CIN mechanisms using the whale cells as more of a null model for human cells and rats as a more similar model. The work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W].

Hexavalent Chromium Decreases Securin Expression and Increases Separase Substrate Cleavage in Human Lung Cells

Jennifer H. Toyoda, Julieta Martino, Rachel Speer, and John Pierce Wise, Sr.

Wise Laboratory of Environmental and Genetic Toxicology, Department of Pharmacology and Toxicology, University of Louisville, Louisville, Ky

Hexavalent chromium [Cr(VI)] is well-known as a lung carcinogen with occupational and environmental exposure risks, but its carcinogenic mechanisms are in need of deeper understanding. Cr(VI) induces chromosome instability (CIN), including changes in chromosome number which may be explained by its ability to cause centrosome amplification. Centrosome amplification is observed in tumors, is associated with aggressive cancers, and is an early event after Cr(VI)-exposure. This study investigates the mechanism of Cr(VI)-induced centrosome disruption in human lung cells. Our hypothesis is Cr(VI) alters activity of separase, the enzyme that cleaves centriole linkers, by decreasing its inhibitor, securin, thus leading to premature centriole disengagement and centrosome amplification. In Cr(VI)-exposed human lung cells, centriole engagement was analyzed by fluorescent immunostaining of centriole markers, CNAP1 and centrin. In normal interphase cells, centriole engagement blocks premature centriole duplication. Our findings show increasing concentrations and prolonged exposure to Cr(VI) increased centriole disengagement in human lung cells. Separase is the key enzyme responsible for cleaving centriole linkers and causing centriole disengagement. Separase activity is tightly controlled by its inhibitor, securin. We show 120 h Cr(VI) exposure caused significant loss of securin protein compared to untreated cells, and furthermore mRNA analysis shows Cr(VI) reduced securin expression. Kendrin is a key protein in stabilizing centriole engagement, and a substrate of separase cleavage. Protein analysis shows full length kendrin is reduced after 120 hours Cr(VI) exposure. Together these data support the conclusion that Cr(VI) decreases securin protein levels by reducing securin expression, which disrupts separase inhibition. Premature centriole disengagement is a potential key to Cr(VI)-induced numerical CIN and may be due to increased kendrin cleavage by aberrant separase. Ongoing studies using securin and separase siRNA knockdown can clarify the role of these proteins in the Cr(VI) carcinogenic mechanism.
This work was supported by NIEHS grant R01ES016893 (J.P.W.) and T32-ES011564 (J.H.T.).

OVSOT 2020 Annual Meeting Posters

10:45 - 12:00 pm Poster presentations and judging

PRESENTATION TITLES, NAME & ABSTRACTS

By Staff, Post-Doctoral, PhD, Masters and Undergraduate

STAFF

Effects of Arylamine N-Acetyltransferase 1 on Tumor Immune Response

Daniel Hodge M.E, Raúl Salazar-González PhD., Kyung Hong PhD., David Hein PhD.

University of Louisville Department of Pharmacology and Toxicology

Introduction: Arylamine N-acetyltransferase 1 (NAT1), an enzyme often upregulated in certain breast cancers subtypes, is associated with superior anchorage-independent growth and bone metastasis. Preliminary results from our lab show that CRISPR/Cas9 NAT1 knockout breast cancer cell lines have increased Major Histocompatibility Complex I (MHC1), as well as accessory molecules Tapasin, TAP1 and TAP2. Altogether, this complex presents peptides from within the cell to cytotoxic T cells, triggering the adaptive immune response and helping it recognize and target cancerous cells.

Objective: The objective of this study is to evaluate the effect of NAT1 on the activation of the immune response in breast cancer.

Methods: Three breast cancer lines, MCF-7 (ER+, PR+, HER2-); MDA-MB-231 (ER-, PR-, HER2-); and ZR-75-1 (ER+, PR+, HER2+) were selected. For each of these cell lines, CRISPR/Cas9 NAT1 KO cell lines were generated and used in further experiments. Cells were grown in appropriate media for 24 hours and then co-cultured with activated Jurkat (T cells) or NK-92 (natural killer) cells in different Effector:Target (E:T) cell ratios for 24 h. After incubation, effector cells were removed and the viability of remaining target cells was quantified.

Results: The co-culture of MCF-7 and MDA-MB-231 parent cells in presence of activated Jurkat cells showed a significant ($p<0.01$) decrease in the cell viability on a 20:1 E:T ratio, resulting in ≈25% less viable cells. Similarly, for both NAT1 KO cell lines the 20:1 E:T ratio showed significant ($p<0.001$) decrease in cell viability, reducing it ≈40% and ≈30% respectively. Cells co-cultured with NK92 cells showed dramatic change in cell survival in the different ratios evaluated ($p<0.0001$). Interestingly, in the NAT1 KO cell lines, the viability was significantly lower ($p<0.0001$) compared to the parent cell line, resulting in 40-80% less viable cells considering the different ratios tested. ZR-75-1 parent cells co-cultured with activated Jurkat or NK92 cells showed significant decrease in cell viability on a 10:1 and 20:1 E:T ratio ($p<0.001$, $p<0.0001$, respectively). No significant ($p>0.05$) effect in cell viability was observed in the ZR-75-1 NAT1 KO cell line.

Conclusions: Our results show that NAT1 is involved in the activation of the immune response in MDA-MB-231 and MCF-7 breast cancer cell lines suggesting that NAT1 has an important role in the immune response to breast cancer.

STAFF

Acetylator Genotype-Dependent Dyslipidemia in Rats Congenic for N-Acetyltransferase 2

Kyung U. Hong, Mark Doll, Angeliki Lykoudi. Raúl Salazar-González, Mariam Habil, Kennedy Walls, Alaa Bakr, Smita Ghare, Shirish Barve, Gavin Arteel, David W. Hein

Departments of Pharmacology & Toxicology and Medicine, and Center for Hepatobiology & Toxicology, University of Louisville School of Medicine, Louisville, KY, USA

Recent reports suggest that arylamine N-acetyltransferases (NAT1 and/or NAT2) serve important roles in regulation of energy utility and insulin sensitivity. We investigated the interaction between diet (control vs. high-fat diet) and acetylator phenotype (rapid vs. slow) using previously established congenic rat lines (in F344 background) that exhibit rapid or slow Nat2 (orthologous to human NAT1) acetylator genotypes. Male and female rats of each genotype were fed control or high-fat (Western-style) diet for 26 weeks. We then examined diet- and acetylator genotype-dependent changes in body and liver weights, systemic glucose tolerance, insulin sensitivity, and plasma lipid profile. Male and female rats on the high fat diet weighed approximately 10% more than rats on the control diet and the percentage liver to body weight was consistently higher in rapid than slow acetylator rats. Rapid acetylator rats were more prone to develop dyslipidemia overall (i.e., higher triglyceride; higher LDL; and lower HDL), compared to slow acetylator rats. Total cholesterol (TC)-to-HDL ratios were significantly higher and HDL-to-LDL ratios were significantly lower in rapid acetylator rats. Our data suggest that rats with rapid systemic Nat2 (NAT1 in humans) genotype exhibited higher dyslipidemia conferring risk for metabolic syndrome and cardiovascular dysfunction.

Post-Doctoral

An endocrinized fibroblast growth factor 1 variant prevents and reverses NAFLD via activating AMPK-mediated pathways

Qian Lin[1], Zhifeng Huang[2], Genxiang Cai[3], Maiying Kong[1], Daniel J. Conklin[1], Paul N. Epstein[1], Kupper A. Wintergerst[1], Moosa Mohammadi[10], Lu Cai[1], Xiaokun Li[2], Yu Li[3], Yi Tan[1]*

[1]University of Louisville, Louisville, KY, USA; [2] Wenzhou Medical University, Wenzhou, China; [3]Chinese Academy of Sciences, Shanghai, China

BACKGROUND AND AIMS: Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disorder. Fibroblast growth factor 1 (FGF1) demonstrated protection against NAFLD in type 2 diabetic and obese mice by an uncertain mechanism. However, its strong mitogenic activity limits its potential clinical application. Our recently engineered FGF1 variant carrying 3 substitutions of heparin-binding sites ($FGF1^{\triangle HBS}$) exhibits greatly reduced proliferative potential, while preserving the full metabolic activity of wild-type FGF1. We investigated the therapeutic activity and mechanism of $FGF1^{\triangle HBS}$ against NAFLD in the present study. APPROACH AND RESULTS: $FGF1^{\triangle HBS}$ administration was effective in 9-month old *db/db* mice with NAFLD; liver weight, lipid deposition and inflammation declined, and liver injury decreased. $FGF1^{\triangle HBS}$ reduced oxidative stress by stimulating nuclear translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) and elevation of antioxidant protein expression. $FGF1^{\triangle HBS}$ also inhibited activity and/or expression of lipogenic genes, coincident with phosphorylation of AMP-activated protein kinase (AMPK) and its substrates. Mechanistic studies on palmitate exposed hepatic cells demonstrated that NAFLD-like oxidative damage and lipid accumulation could be reversed by $FGF1^{\triangle HBS}$. In palmitate-treated hepatic cells, AMPK inhibition by pharmacological agent or siRNA abolished $FGF1^{\triangle HBS}$ benefits on both oxidative stress and lipid metabolism. Further support of these *in vitro* findings is that liver-specific AMPK knockout abolished therapeutic effects of $FGF1^{\triangle HBS}$ against high-fat/high-sucrose diet-induced hepatic steatosis. Moreover, $FGF1^{\triangle HBS}$ improved high-fat/high-cholesterol diet-induced steatohepatitis and fibrosis in apolipoprotein E knockout mice. $FGF1^{\triangle HBS}$ decreased the liver weight, lipid deposition, fibrosis, inflammation and ameliorated the liver injury, coincident with the upregulation of the phosphorylation of AMPK and its substrate. CONCLUSIONS: These findings indicate that $FGF1^{\triangle HBS}$ is effective for preventing and reversing liver steatosis and steatohepatitis and acts by activation of AMPK. $FGF1^{\triangle HBS}$ might be a therapeutic approach for the treatment of NAFLD without promoting undesired tissue proliferation.

Post-Doctoral

CD163 protects against O3-induced oxidative stress and pulmonary injury

Katelyn Dunigan Russell[1], Sky W. Reece[2], Michael Yaeger[1], Elizabeth A. Browder[2], Myles X. Hodge[2], Bin Luo[2], Brita J. Kilburg-Basnyat[2], Hongmei Zhu[3], Timothy J. McMahon[3], Robert M Tighe[3], Kymberly M. Gowdy[1]

[1]Ohio State University, Columbus, OH, [2]East Carolina University, Greenville, NC[3], Duke University Medical Center, Durham, NC.

Epidemiological studies have reported that exposure to elevated ambient concentrations of ozone (O3) is associated with adverse pulmonary and cardiovascular outcomes. The underling biological mechanisms mediating O3 associated adverse health effects are unknown. O3 induces oxidative stress (OS) in the lung leading to pulmonary inflammation and injury. Pulmonary inflammation and injury correlates with increased levels of damage associated molecular patterns (DAMP). We have found that O3 exposure is associated with increased levels of the DAMP cell free hemoglobin (CFH). CFH is endocytosed by CD163, a scavenger receptor exclusively expressed on macrophages/monocytes; the uptake results in the up-regulation of heme oxygenase 1 (HO-1). We hypothesize O3 -induced oxidative stress increases CFH in the airspace and CD163 is critical for protecting against this DAMP induced injury. C57BL/6J (WT) and CD163-/- male mice were injected with vehicle or 100 mg/kg of N-Acetyl-Cysteine (NAC) and then, 1h later, exposed to either filtered air (FA) or 1ppm O3 for 3h. BAL and blood samples were obtained 6h or 24hr post exposure and bronchoalveolar lavage (BAL) cell counts, CFH, and total protein were measured. Real time PCR was used to measure HO-1, proinflammatory cytokine, and chemokine levels in lung tissue. After O3 exposure, WT mice had a significant increase in CFH in the BAL which was augmented in O3 exposed CD163-/- mice. O3 exposed CD163-/- mice had greater pulmonary inflammation and injury compared to WT mice as evidenced by increased BAL neutrophils, macrophages, and total protein. CD163-/- mice exposed to O3 had significantly lower levels of HO-1 when compared to WT controls. In WT mice, NAC pretreatment did not alter O3-induced pulmonary inflammation or injury whereas NAC pretreatment of CD163-/- mice significantly decreased BAL protein. Our findings suggest the mitigation of O3-induced oxidative stress is dependent on CD163 due to its ability to clear excessive DAMPs such as CFH. Future studies will examine the underlying mechanism of CFH-augmented OS and its relationship to monocyte/macrophage inflammation.

Post-Doctoral

Characterizing age-associated microbial dysbiosis in people living with HIV (PLWH)

Richa Singhal, S. Ghare, M. Vadhanam, C. Tirumala, S. Gautam, C.J. McClain, and S. Barve

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Background

Emerging evidence shows that HIV-infection leading to chronic immune activation coupled with a lack of anti-inflammatory response results in accelerated aging in humans. Both aging and HIV infection are independently linked with microbial dysbiosis leading to immune activation. However, there is a limited understanding of qualitative and quantitative determinants of dysbiosis due to aging in PLWH. Hence a pilot study was conducted to investigate the dysbiotic features of the microbiome relevant to pathogenic effects of aging in PLWH

Method

Fecal specimens, plasma and relevant clinical parameters of PLWH (n=31) with known age (50-70) were obtained. Metagenomic analysis of the V4 region of the 16S rRNA gene were performed using the Illumina MiSeq platform. Cytokines were measured using Meso Scale Discovery platform. Statistical analyses included LEfSe, Mann Whitney test and Spearman correlations

Objective

Characterize gut microbial dysbiosis associated with HIV-infection and aging in PLWH

Result

Electrochemiluminescence analysis showed a significant increase of proinflammatory cytokines including IFN-g, IL-13, IL-2, and IL-6 in the age group (60-69) in comparison to age group (50-59). Taxa analysis revealed that microbial dysbiosis associated with aging in PLWH is characterized by a significant reduction of the Firmicutes/Bacteroidetes (F/B) ratio and beneficial butyrate-producing family Lachnospiraceae. Moreover, butyrate producers Acidaminococcaceae, Veillonellaceae, Erysipelotrichaceae, and the butyrate-producing families as a collective were significantly reduced in highest age group (60-69). A decrease in the F/B ratio and butyrate producers were correlated with an increase in IFABP and proinflammatory cytokines (TNF-alpha, IL8, IL2, IFN-g, etc.). These observations indicate that age is a significant negative co-factor affecting the loss of butyrate-producing bacteria leading to an increase in intestinal permeability, microbial translocation and consequent systemic inflammation and immune dysfunction in PLWH

Conclusion

Aging is a significant negative co-factor in HIV pathogenesis affecting gut microbial dysbiosis marked by a significant loss of butyrate producing bacterial communities in PLWH

Doctoral Graduate Student

Metabolism, morphological effects, and behavioral alterations following a developmental atrazine exposure in zebrafish

[1]Janiel K. Ahkin Chin Tai, [1,2]Katharine A. Horzmann Ph.D., [1]Jennifer L. Freeman, Ph.D.

[1]School of Health Sciences, Purdue University, West Lafayette, IN, [2]Department of Pathobiology, Auburn University, Auburn, AL

Atrazine (ATZ) is a triazine herbicide that is commonly used on crops in the midwestern United States and globally. ATZ contaminates potable water sources, so the US EPA has set the regulatory level at 3 parts per billion (ppb;ug/l) in drinking water. Depending on the time of year and sampling location, water sources often exceed this limit. ATZ has a long half-life and has also been implicated as an endocrine disrupting chemical in multiple species. The current study used a biomedically relevant model, the zebrafish, to test the hypothesis that developmental ATZ exposure generates metabolites similar to those found in mammals and alters morphology and behavior in developing larvae. Adult AB zebrafish were bred, embryos were collected, and exposed to 0, 0.3, 3 or 30 ppb ATZ from 1 to 120 hours post fertilization (hpf). Targeted metabolomics found that zebrafish produce the same major metabolites as mammals (desethyl atrazine, deisopropyl atrazine, and didealkyl atrazine) with ATZ exposure. Morphology measurement do not show any significant increase in mean head width, head length, or brain length at 120 hpf ($p>0.05$). The cumulative visual motor response test detected hyperactivity in the 0.3 ppb treatment groups with distance moved ($p<0.05$), time spent moving ($p<0.05$), and velocity ($p<0.05$). Phasic behavioral analysis detected hyperactivity in the light phases for the 0.3 ppb treatment group for total distance and time spent moving and hypoactivity in 30 ppb treatment group in the dark phases ($p<0.05$). These findings indicate that ATZ exposure during early development generates metabolite profiles similar to mammals and leads to morphology and behavioral alterations supporting ATZ as a developmental toxicant.

Doctoral Graduate Student

Do Aldehydes in Conventional Tobacco Smoke Stimulate Platelet-Mononuclear Cell Aggregation In Vivo?

Andre Richardson[1,2] and Daniel J. Conklin, Ph.D.

[1]Department of Pharmacology & Toxicology, [2]American Heart Association - Tobacco Regulation Center, [3]Diabetes and Obesity Center, [4]Division of Cardiovascular Medicine
[1-4]University of Louisville

Conventional cigarette smoking is the single largest risk factor for cardiovascular disease (CVD). Researchers show a potential association between aldehydes in tobacco-derived aerosols from mainstream cigarette smoke (MCS) and cardiotoxicity by damaging blood vessel endothelium. This can lead to myocardial infarctions or stroke due to blood clots (a pro-thrombotic event). However, the mechanisms by which levels of harmful and potentially harmful constituents (HPHCs) such as aldehydes induce cardiotoxicity are not well known. The purpose of this research was to examine the effects of saturated and unsaturated aldehydes as well as MCS on platelet-mononuclear cell aggregates (PMAs) -- a biomarker of thrombosis in vivo. To address these effects we used flow cytometry. Adult male and female C57BL/6 mice underwent acute exposures (6h/d, 4d) to filtered air (control), acetaldehyde (5 ppm), crotonaldehyde (3 ppm), formaldehyde (2 & 5 ppm), or MCS (3R4F; 12 cigs/d). Additionally, we performed a 2-week exposure of HEPA-filtered air (control), acetaldehyde (5 ppm), or formaldehyde (5 ppm) to C57BL/6 male mice for two weeks (6h/d) as well as a 12-week exposure to crotonaldehyde (1 ppm) for 12 weeks (6h/d). After final exposure, mice were euthanized and peripheral blood was used for analysis. PMA levels were measured using flow cytometry by monitoring for the number of aggregates double positive for CD41+ (platelet marker) and CD45+ (leukocyte marker). IgG isotype negative controls were used to establish proper gating. These data indicate that the PMA levels in male mice exposed acutely to acetaldehyde (5 ppm) or formaldehyde (2 & 5 ppm) were unchanged compared with control. We observed significant increases of PMAs in male mice exposed to MCS (3.7±1.0% vs. 2.0±0.81%) and crotonaldehyde (6.0±1.0% vs.12.0±2.0%) compared with their respective controls. Chronic exposure to acetaldehyde, formaldehyde, or crotonaldehyde had no effect on PMA levels. In females exposed to acetaldehyde (5 ppm) or formaldehyde (2 & 5 ppm), PMA levels were also unchanged. The FDA seeks to regulate the levels at which these aldehydes in tobacco derived aerosols of MCS exert toxicity. In addition to discovering whether individual aldehydes stimulate thrombotic responses, our research will provide data regarding the levels of aldehydes that trigger such events; these data are useful to the FDA when regulating the HPHCs in tobacco product aerosols. Thus, this research serves to assist in determining cardiotoxic effects of these aldehydes that way the FDA regulates their output in the aerosols. Furthermore, this research may also aid in understanding potential mechanisms of aldehyde-induced thrombogenesis.

Doctoral Graduate Student

Sex Differences in Pulmonary Eicosanoid Metabolism in Response to Ozone Exposure

Michael Yaeger[1,2], Sky W. Reece[2], Brita J. Kilburg-Basnyat[2], Myles Hodge[2], Christine Psaltis[2], Bin Luo[2], Michael Armstrong[3], Nichole Reisdorph[3], Espen E. Spangenburg[4], Johanna L. Hannan[4], Robert M. Tighe[5], Saame Raza Shaikh[6], Kymberly M. Gowdy[1,2]

[1]Ohio State University, Columbus, OH 43210; [2] Department of Pharmacology and Toxicology, ECU, Greenville, NC; [3]School of Pharmacy, UC Denver, Denver, CO; [4]Department of Physiology, ECU, Greenville, NC; [5]Department of Medicine, Duke University, Durham, NC; [6]Department of Nutrition, UNC at Chapel Hill, NC.

Ozone (O3) is a criteria air pollutant known to increase the morbidity and mortality of cardiopulmonary diseases. Cardiopulmonary diseases are induced, in part, by a pulmonary inflammatory response characterized by recruited pulmonary immune cells, increased pro-inflammatory cytokines, and altered production of pro-inflammatory and pro-resolving lipid mediators. Recent evidence has demonstrated there are sex-dependent differences in the O3-induced pulmonary inflammatory response. However, if this dimorphic response is driven by pulmonary lipid mediators has yet to be examined. Therefore, we hypothesized there are sex-dependent differences in lipid mediator production following O3 inhalation. Male and female C57BL/6J mice were exposed to 1 ppm O3 for 3 hours and were necropsied 6 and 24 hours following exposure. Lung lavage was collected for cell differential and protein analysis, and lung tissue was collected for RNA analysis, lipid mediator and fatty acid quantitation, and immunohistochemistry. Comparable to other studies, O3 exposed female mice had increased airspace neutrophilia, and Cxcl1 and Cxcl2 expression. Additionally, females had significant increases in several pro-inflammatory lipid mediators including 12S-HETE, PGF2α, and PGE2 following O3 exposure. Specialized pro-resolving mediators (SPMs) 14(S)-HDHA, 17(S)-HDHA, PDX, and RvD5 were also significantly increased in female lung tissue when compared to males post exposure. Likewise, precursor fatty acids arachidonic acid (AA) and DHA were increased in female lung tissue at baseline and following O3 exposure. Curiously, these findings were independent of ovary producing hormones, as evidenced by examining ovariectomized female mice. Taken together these data indicate that O3 drives an augmented pulmonary inflammatory response in females that coincides with increased fatty acids in lung tissue, pro-inflammatory lipid mediators, and SPMs.

Masters

MiR-186 overexpression exacerbates the arsenic-induced chromosomal instability associated with skin carcinogenesis

Angeliki Lykoudi, Ana P. F. Cardoso, Sandra S. Wise and J. Christopher States

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Background: Chronic arsenic exposure through drinking water is a global health issue, affecting > 200 million people. Arsenic is a group I human carcinogen and clastogen causing chromosomal instability (CIN). Skin is the primary target organ for arsenic toxicity. miRNA dysregulation and CIN are suggested mechanisms of arsenic carcinogenesis. Preliminary data indicate that miR-186 is overexpressed in arsenic-induced squamous cell carcinoma relative to premalignant hyperkeratosis. Predicted targets of miR-186 are cell cycle regulators, thus overexpression of miR-186 potentially leads to CIN and aneuploidy, features of cancer. We showed that overexpressing miR-186 in HaCaT cells increased numerical and structural chromosomal abnormalities. Hypothesis: miR-186 overexpression drives malignant transformation of HaCaT cells by induction of CIN and potentially accelerates arsenic-induced transformation. Methods: Stable clones of HaCaT transfected with pEP-miR-186 expression vector or empty vector were maintained under puromycin selection. Selected clones exposed to 0 or 100 nM NaAsO2 were cultured for 29 weeks. Anchorage independent growth was tested in soft agar colony formation assay. Giemsa banding was used to produce karyotypes and cytogenetic analysis for chromosomal aberrations was performed. Results: HaCaT overexpressing miR-186 and exposed to NaAsO2 showed increased growth ability in agar in 12 weeks in contrast to vector control cells exposed to NaAsO2. This group of cells also showed additional material of chromosome 1 and 20 in all cells analyzed and significant number of losses of chromosomes 13, 14, 15 and 16. The levels of E-cadherin were also reduced in all arsenite-exposed cells in 29 weeks compared to 12 weeks. Discussion: These results suggest that miR-186 overexpression exacerbates the arsenite-induced CIN and potentially is associated with accelerated skin carcinogenesis. The authenticity of the cells was validated by the presence of reported marker chromosomes. Only one clone and one timepoint were analyzed for each group and the presence of these karyotypic changes might be a feature of this specific clone and not an effect of arsenic toxicity. Thus, future research using additional clones is warranted.

Masters

Depletion of arylamine N-acetyltransferase 2 results in dysregulation of gluconeogenic and lipogenic genes in HepG2 hepatocellular carcinoma cell line

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Arylamine *N*-acetyltransferase 2 (NAT2) is a polymorphic enzyme involved in phase II metabolism of hydrazine derivatives and aromatic amine compounds. Recent studies have described a novel link between *NAT2* genetic polymorphisms and insulin resistance. However, the precise mechanism remains unknown, and so far, all studies investigating this link have been performed in murine models. The ability to switch the gene program in response to fed vs. fasted states is critical for normal glucose and lipid homeostasis in liver. In humans, NAT2 is mainly expressed in liver and GI tract. To investigate the role of NAT2 in regulation of glucose and lipid metabolism in cells of human liver origin, we stably silenced NAT2 in HepG2 (hepatocellular carcinoma) cells using lentivirus expressing shRNAs. Depletion of NAT2 by shRNAs was confirmed by measuring *in situ* NAT2 activity for sulfamethazine. The control and NAT2 shRNA cells were subjected to two conditions of excess energy (i.e., low vs. high glucose or BSA vs. high free fatty acid [FFA]). Transcripts of genes involved in gluconeogenesis (*G6PC, PCK1*) and lipogenesis (*FASN, SREBF1*) were then measured using qRT-PCR. Even in the absence of high glucose or high FFA treatment, expression of both gluconeogenic and lipogenic genes were significantly reduced in NAT2 shRNA cells, compared to the control shRNA cells. In response to excess nutrients, NAT2 shRNA cells showed significant alterations in the pattern and magnitude of gene expression. The high FFA treatment downregulated *FASN* and *SREBF1* transcripts in control cells. NAT2 shRNA cells, however, showed increases in expression of the lipogenic genes under the high FFA condition. In addition, *PCK1* (PEPCK) mRNA was significantly upregulated in high FFA-treated NAT2 shRNA cells, while the treatment did not affect *PCK1* expression in control cells. These results suggest that depletion of NAT2 leads to defects in glucose and lipid production in HepG2 cells at both normal and excessive levels of nutrients and that hepatocytes expressing low NAT2 activity may be less able to effectively respond to the switch between fed and fasted states. Further investigation of the dysregulation of gluconeogenic and lipogenic genes in NAT2 knockdown cells will aid in understand how NAT2 influences hepatic glucose and lipid homeostasis as well as insulin sensitivity

Masters

Rad51 Paralogs Are Key Targets for Loss of Homologous Recombination Repair in Metal Carcinogenesis

Aggie Williams, Rachel Speer, Cynthia Browning, Idoia Meaza, Jennifer Toyoda and John Pierce Wise, Sr

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Lung cancer is a major cause of cancer death around the world and recovery rates have not improved for decades. A major factor in the lack of progress is that lung cancer is often dismissed and overlooked as a disease simply caused by smoking and solved by smoking cessation. However, smoking cannot account for substantial amounts if the disease, and other agents are clearly major causes of lung cancer. Metals are a major group of causative lung cancer agents and millions of people are exposed. Metals are poor base mutagens, but potently damage chromosomes indicating chromosome instability (CIN) is a major factor in their carcinogenic mechanism. We focus on hexavalent chromium [Cr(VI)] as a representative metal to investigate the underlying mechanisms of metal-induced CIN, as millions are exposed to Cr(VI) and Cr(VI) has the best characterized CIN outcomes compare to many metals, thus it offers a direct path forward in understanding these mechanisms. Homologous recombination repair is a crucial DNA repair pathway that prevents CIN by repairing DNA double-strand breaks. RAD51 is the key effector protein in homologous recombination repair and the RAD51 paralogs (RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3) act as important orchestrators of RAD51 function. Cr(VI) induces DNA double strand breaks and targets RAD51 in human lung cells. We hypothesized that Cr(VI) targets the RAD51 paralogs, a key regulator of RAD51. We started with RAD51C and RAD51D investigating the effects of acute (24h) and prolonged (120h) Cr(VI) exposure. Using immunofluorescence to measure DNA repair function, we showed acute Cr(VI) exposure induces the homologous recombination repair response manifested as increased RAD51 foci and RAD51C foci in human lung cells. In contrast longer exposures show both proteins are inhibited. Remarkably, Cr(VI) reduced RAD51D foci formation after both acute and prolonged exposures, suggesting RAD51D may be a key target in Cr(VI)-reduced RAD51 function and homologous recombination repair inhibition. Future work will explore this hypothesis. This work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W].

Undergraduate

Developmental behavioral alterations following lead (Pb exposure) in the zebrafish model system

Jenny Chen, Keturah Kiper, Jennifer L. Freeman

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Lead (Pb) is a toxic heavy metal of concern that can be found in drinking water, dust, and soil. Environmental exposure to lead has been associated with neurological alterations in both adults and children. Numerous studies have suggested adverse health outcomes caused by the neurotoxic effects of lead and its ability to interfere with many physiological processes in the central nervous system. For example, epidemiological studies indicate lead can induce neurobehavioral alterations and cognitive impairments that result in lowered intelligence quotient and increased risk for attention deficit hyperactivity disorder (ADHD), a mental health disorder caused by hyperactive and impulsive behavior. This study used the zebrafish model to investigate the developmental toxicity effects of exposure to nonlethal concentrations of lead from 1 through 120 hours post fertilization (hpf). The concentrations used were 0 ppb, 10 ppb, 50 ppb, 100 ppb, 500 ppb, and 1,000 ppb. The visual motor response test was used to assess toxicity effects of lead through changes in behavior and locomotion. Phasic data was collected and analyzed using a repeated measures ANOVA for alternating dark and light phases with a total of 5 phases, each lasting for 10 minutes. Phasic behavior data showed hyperactivity through increased velocity and distance moved in all of the dark phases for the 10 ppb treatment group. Larvae in the 50 ppb treatment group showed hyperactivity in the second light phase through increased velocity, time spent moving, and distance travelled. Hypoactivity, depicted through decreased velocity, distance moved, and time spent moving occurred in the 100 ppb treatment group in the first light phase. Larvae in the 500 ppb treatment group only exhibited a decreased time spent moving in the first two dark phases and first light phase. The 1000 ppb treatment group spent less time swimming only in the first dark phase. These findings indicate zebrafish larvae that were exposed to lead early in development display various changes in behavior and locomotive activity dependent on lead exposure concentration. Overall, increased behavioral and locomotor implications were observed at the lower lead exposure concentrations in this study. Changes in behavior may be indicative of improper central nervous system development, specifically the sensory-motor pathways in the brain as observed in other studies.

Undergraduate

Assessing Adult Learning and Memory in Three Genotypes of Mice Exposed to Benzo[a]Pyrene During Early Brain Development

Katelyn Clough, Emma Foster, Jayasree Mullaguru, Emma DeBurger, Kayla Jenkins, Victoria Ferguson, Tyler Forrest, Angela Kyntchev, Diarra Niang, and Christine Perdan Curran

Northern Kentucky University Department of Biological Sciences, Highland Hts KY

Benzo[a]pyrene (BaP) is a carcinogenic polycyclic aromatic hydrocarbon commonly found in traffic-related air pollution, tobacco smoke, and grilled foods. BaP is linked to learning deficits and to neurodevelopmental delays in human and animal studies. We are using a mouse model to determine if genetic differences increase susceptibility to BaP exposure during early brain development. Mice with variations in the aryl hydrocarbon receptor, lacking the CYP1A2 metabolic enzyme and wild type control mice were exposed to 10mg/kg/day BaP from gestational day 10 (GD10) through weaning at postnatal day 25 (P25). A battery of cognitive and motor function tests were performed when the mice reached early adulthood (P60). We used Novel Object Recognition and Morris Water Maze to assess non-spatial visual learning and memory. Preliminary results from the first five behavioral cohorts will be presented. BaP-treated knockout mice spent less time exploring the novel object, but the differences were not significant ($P = 0.077$). BaP-treated *Ahr*^{b}*Cyp1a2(-/-)* mice had significantly longer path lengths on Days 3, 5 and 6 in the Acquisition Phase of Morris Water Maze ($P < 0.05$). BaP-treated mice had significantly impaired reference memory in the Acquisition and Shift-reduced Probe trials ($P < 0.05$). We will test additional cohorts until we have tested $n \geq 16$ litters per group.

Undergraduate

Using High-Performance Liquid Chromatography to Measure the Effects of Genetic Variation on Dopamine and Serotonin Levels Following Prenatal Exposure to Benzo[a]pyrene

Emma Foster, Katelyn Clough, Lisa Massie, and Christine Perdan Curran

Northern Kentucky University Department of Biological Sciences, Highland Heights, KY

Traffic-related air pollution consists of particulates, gases, and organic molecules like polycyclic aromatic hydrocarbons (PAHs). This pollution is known to increase the risk of lung cancer and cardiopulmonary issues, but TRAP has also been found to have detrimental effects on the brain. Recent studies have found that aryl hydrocarbon receptor (AHR) agonists, like PAHs, have neurotoxic effects, especially during development. Exposure to benzo[a]pyrene (BaP), a PAH and model neurotoxicant for TRAP, has been linked to deficits in learning and memory and dopaminergic pathways in animal studies. Based on BaP metabolism, it has been suggested that some individuals may be more susceptible to TRAP exposure. Our study aims to determine the effects of genetic variation on BaP developmental neurotoxicity, including neurotransmitter levels in the brains of adult mice exposed during pregnancy and lactation. We use mice with genetic differences in the AHR and CYP1A2 to model human genetic variation. Pregnant dams were treated with 10mg/kg/day BaP in corn oil-soaked cereal or the corn oil vehicle from gestational day 10 to postnatal day 25. One male and one female per litter were assigned to behavioral testing. Following behavioral testing at postnatal day 120, striatum, hippocampus, prefrontal cortex, and hypothalamus were collected. Dopamine and serotonin levels were measured using High-Performance Liquid Chromatography with Electrochemical Detection. In our preliminary analysis, we found increased levels of dopamine in the striatum of BaP-treated mice ($P = 0.074$) and lower levels of serotonin ($P = 0.099$), but the differences didn't reach statistical significance. There was a significant gene x treatment interaction in the hippocampus with BaP-treated wild type *Ahr^{b}Cyp1a2(+/+)* and poor affinity *Ahr^{d}Cyp1a2(-/-)*mice having lower dopamine levels compared with corn oil-treated controls. In contrast, BaP-treated high affinity *Ahr^{b}Cyp1a2(-/-)* showed the greatest changes in the prefrontal cortex with significantly decreased dopamine and serotonin levels ($P < 0.05$). Together, these findings suggest that all three genotypes show some susceptibility to developmental BaP exposure.

Undergraduate

The effects of environmental copper exposure on the behavior and morphology of developing zebrafish

Christina Kaucic, Keturah Kiper, and Jennifer L. Freeman

Purdue University, 610 Purdue Mall, West Lafayette, IN 47907

Copper is an essential metal that is key in many metabolic functions and is a cofactor in many enzymes. Excess copper has been associated with fatigue, weakness, and memory loss as well as with neurological disorders and some cancers in humans. Similarly, excess copper has been shown to damage visceral organs and produce abnormal behaviors in multiple fish species including the zebrafish who has a high degree of genome sequence homology when compared to humans. For this reason, the zebrafish is an advantageous model for the study of copper toxicity. In this study, we assessed survival and then behavioral and morphological changes at sub-lethal concentrations in developing zebrafish with exposure spanning 1 to 120 hours post fertilization (hpf). Exposure concentrations included 0, 13, 130, and 1300 ppb to span the current US EPA regulatory level in drinking water for the survival analyses and revised to sub-lethal concentrations of 0, 13, and 130 ppb for the behavior and morphology assessments. We hypothesized that zebrafish exposed to higher levels of copper during embryogenesis would show signs of increased physiological and behavioral stress as well as abnormalities in morphology. Copper caused mortality at 1300 ppb and further experiments focused on sub-lethal concentrations. Behavioral studies revealed decreased time spent moving as well as decreased counterclockwise rotation frequency at 130 ppb ($p<0.05$). In morphological analysis, exposed larvae exhibited significantly decreased head width, head length, total length, brain length, and eye diameter at 130 ppb ($p<0.05$). Developmental exposure to copper produces dysfunctional locomotor behavior and abnormalities in morphology in zebrafish at concentrations lower than the regulatory concentration in US drinking water indicating species sensitivity. Future studies will focus on copper exposure and alterations in gene expression with a specific interest in studying myelination in both the peripheral and central nervous systems and neural development pathways.

Tox-on-the-Clock

1:00 - 2:00 pm

PRESENTATION TITLES, NAME & ABSTRACTS

An Alkali Metal-Based Au MPCs Chemiresistor Sensor Array for Sensing Aromatic VOCs

Prasadanie K. Adhihetty,[a] Sujoy Halder,[b] Xiao-An Fu,[b,*] and Michael H. Nantz[a]

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We aim to develop chemiresistive sensors to detect trace levels of aromatic volatile organic compounds (VOCs) in outdoor and indoor air. Many aromatic VOCs, such as benzene, toluene, ethylbenzene and xylene (BTEX), have harmful effects on human health. Consequently, methods have been developed to detect BTEX in ppb to ppt levels. However, the detection and analysis of these aromatic VOCs without analyte preconcentration has not yet been reported. Our interest is to design a sensor comprised of metal ion-functionalized thiol ligands coated on gold monolayer protected clusters (Au MPCs) as a chemiresistor for detecting BTEX VOCs at room temperature with high sensitivity and selectivity by accommodating microelectromechanical system (MEMS) technology. Aromatic compounds contain conjugated π systems that interact with electron deficient elements through cation-π interactions. In some circumstances, this specific noncovalent interaction can be stronger than hydrogen bonding. In this study, we have synthesized nanoparticles that feature alkali metal ions bound to the surface of thiol ligand-coated Au MPCs. The Au MPCs are deposited within interdigitated electrodes fabricated by MEMS technology to create a chemiresistor that senses trace levels of benzene toluene, ethylbenzene, and xylene, presumably through magnified cation-π interactions at the surface of the Au MPCs. Herein, we report our initial studies on harnessing cation-π interactions for sensing aromatic VOCs. Details on syntheses of the chelating thiol ligands and Au MPCs will be presented as well as the methods of alkali metal surface functionalization, particle characterization, and chemiresistor responses. N-Boc protected short alkane chain (6C) and long alkane chain (11C) aminooxy thiols were synthesized and used to prepare thiol ligand-coated Au MPCs with ~2 nm particle diameter via the two phase Brust-Schiffrin method. N-Boc deprotection was followed by click chemistry to attach ester-functionalized surface groups that subsequently were used to incorporate alkali metal ions on the particle surface. Finally, we will present the aromatic VOCs sensing ability of chemiresistors derived from Li+, Na+ and K+ short-chain (6C) and long-chain (11C) thiol-coated Au MPCs.

Assessing Adult Learning and Memory in Three Genotypes of Mice Exposed to Benzo[a]Pyrene During Early Brain Development

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Benzo[a]pyrene (BaP) is a carcinogenic polycyclic aromatic hydrocarbon commonly found in traffic-related air pollution, tobacco smoke, and grilled foods. BaP is linked to learning deficits and to neurodevelopmental delays in human and animal studies. We are using a mouse model to determine if genetic differences increase susceptibility to BaP exposure during early brain development. Mice with variations in the aryl hydrocarbon receptor, lacking the CYP1A2 metabolic enzyme and wild type control mice were exposed to 10mg/kg/day BaP from gestational day 10 (GD10) through weaning at postnatal day 25 (P25). A battery of cognitive and motor function tests were performed when the mice reached early adulthood (P60). We used Novel Object Recognition and Morris Water Maze to assess non-spatial visual learning and memory. Preliminary results from the first five behavioral cohorts will be presented. BaP-treated knockout mice spent less time exploring the novel object, but the differences were not significant ($P = 0.077$). BaP-treated *Ahr^{b}Cyp1a2(-/-)* mice had significantly longer path lengths on Days 3, 5 and 6 in the Acquisition Phase of Morris Water Maze ($P < 0.05$). BaP-treated mice had significantly impaired reference memory in the Acquisition and Shift-reduced Probe trials ($P < 0.05$). We will test additional cohorts until we have tested $n \geq 16$ litters per group.

Differences in Acetyl coenzyme A affinity between N-acetyltransferases NAT1 and NAT2

Mariam R. Habil, Mark A. Doll, David W. Hein

Department of Pharmacology and Toxicology, University of Louisville

Arylamine N-acetyltransferases or NATs are xenobiotic-metabolizing enzymes that catalyze the acetylation reaction by which an acetyl group from the cofactor acetyl coenzyme A (acetyl CoA) is transferred to substrate. Two N-acetyltransferase proteins (NAT1 and NAT2) are expressed in humans. NAT1 is found in most human tissues while NAT2 is mainly found in liver and gastrointestinal tract. NATs have an important role in detoxification and/or activation of different carcinogens such as 4-aminobiphenyl (ABP). Exposure to ABP occurs in the chemical, dye, and rubber industries as well as in hair dyes, paints, and cigarette smoke. Acetyl coenzyme A has been shown to protect NAT1 from oxidative inactivation, and NAT1 levels have been shown to modify endogenous acetyl CoA levels in breast cancer cells. Both human NAT1 and NAT2 catalyze the N-acetylation of ABP. In the present study, we investigated the role of acetyl CoA in ABP N-acetylation in Chinese hamster ovary (CHO) cells that were stably transfected with either human NAT1*4 or NAT2*4 (the reference or rapid acetylators). In vitro ABP N-acetyltransferase assays were done at 300 µM ABP and different acetyl CoA concentrations (500- 5000 µM). N-acetyl ABP was measured by high pressure liquid chromatography (HPLC) and acetyl CoA Michaelis-Menten kinetic values were determined and compared between NAT1 and NAT2. The apparent acetyl CoA Km for NAT2*4 was significantly higher (17 -fold) than that of NAT1*4 ($p < 0.001$). The apparent acetyl CoA Km values were 95.1 ± 26.5 µM and 1615 ±124 µM for human NAT1*4 and NAT2*4, respectively reflecting higher affinity of NAT1*4 towards acetyl CoA. Further studies will be done to determine and compare affinity of acetyl CoA for NAT1 and NAT2 genetic variants.

Using the zebrafish model system to study the developmental toxicity of arsenic and lead mixtures

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The heavy metals arsenic (As) and lead (Pb) are environmental pollutants, are often found in common sites, and are linked to adverse health effects, including cardiovascular toxicity and neurotoxicity. A variety of studies have determined developmental toxicity and biomarkers of low dose exposure to each of these metals, but there are limited studies on the binary mixture. This study evaluated the interaction between As and Pb to determine what type of interaction occurs at lethal and sub-lethal concentrations using the zebrafish model. The concentration addition (CA) model was applied to survival data to determine if the type of interaction between Pb and As is additive due to shared neurotoxicity and vascular toxicity pathways. Metal exposures were from 1-hour post-fertilization (hpf) through 120 hpf. As concentrations were 0–10E6 µM and Pb concentrations were 0–480 µM in the survival study. The LC_{25}, LC_{50}, and LC_{75} values at 120 hpf from the single metal exposures were used to determine the mixture concentrations for three separate mixture experiments. The pH for all treatments was adjusted to 6.5 to avoid precipitation of Pb in solution. The survival data indicated an additive non-interaction effect was occurring at these concentration ranges. To further examine the impact of this metal mixture on development, behavior and morphological alterations were evaluated at sub-lethal concentrations of 10 and 100 ppb As (0.133 and 1.33 µM) and Pb (0.048 and 0.48 µM) individually or in mixtures. Zebrafish larvae exposed to 10 ppb As (0.133 µM) exhibited hyperactivity in all dark phases for the distance moved, time moving, and velocity, while those exposed to 10 ppb Pb (0.048 µM) only showed an increase in distance moved and velocity in the first dark phase. The 10 ppb As and Pb mixture was found to have an intermediate impact with increased time moving in all dark phases and increased distance moved and velocity only in the first dark phase. In contrast, hyperactivity was observed only in the 100 ppb mixture (1.33 µM As and 0.48 µm Pb) in the last two dark phases for time moving and in the last dark phase for the distance moved. No significant behavioral alterations occurred in the single 100 ppb treatments. A decrease in mean brain length and brain length ratio to the total length in the 10 ppb mixture (0.133 µM As and 0.48 µM Pb) was observed with no significant morphological changes observed for head length, head width, or total length. Overall, this study serves as a foundation for future studies at lower mixture concentrations.

Whale Cells Are Resistant to Cr(VI)-Induced Loss of Homologous Recombination Repair

Haiyan Lu, Sandra S. Wise, Jennifer H. Toyoda, Rachel M. Speer, Alicia Bolt, John Pierce, Wise, Sr.

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Particulate hexavalent chromium [Cr(VI)] is a well-established human lung carcinogen, but the mechanism for Cr(VI)-induced cancer is uncertain. Chromosome instability (CIN) is a hallmark of lung cancer and is considered a major factor in Cr(VI)-induced lung cancer. Structural CIN can result from unrepaired DNA double strand breaks. Homologous recombination (HR) repair protects against these breaks. In human lung cells, we found Cr(VI) induces DNA double strand breaks while simultaneously inhibiting DNA double strand break repair, resulting in CIN. Whales face long-term exposure to Cr(VI) and accumulate Cr in their tissues, but appear to have a low incidence of cancer. Thus, to further explore the mechanism of Cr(VI)-induced lung cancer, we tested the hypothesis that whales are resistant to Cr(VI)-induced CIN. We measured the ability of Cr(VI) to induce DNA double strand breaks, HR repair, and chromosome damage in whale lung cells. We discovered Cr(VI) induces DNA strand breaks in whale cells, but whale cells avoid repair inhibition and maintain their HR repair response. Consequently, the amount of chromosomal damage was greatly reduced with no apparent CIN. By contrast, rats are a common model to study Cr(VI) though no studies have considered HR, DNA double strand breaks or HR repair. To determine if rats respond similarly to humans or more like whales, we compare DNA double-strand breaks and HR repair in rat lung cells and confirmed that Cr(VI)-induced genetic instability in the rat is similar to humans. Thus, future studies will determine the underlying CIN mechanisms using the whale cells as more of a null model for human cells and rats as a more similar model. The work was supported by the National Institute of Environmental Health Sciences [ES016893 to J.P.W].

MiR-186 overexpression exacerbates the arsenic-induced chromosomal instability associated with skin carcinogenesis

Angeliki Lykoudi, Ana P. F. Cardoso, Sandra S. Wise and J. Christopher States

Dept. Pharmacology and Toxicology, University of Louisville, Louisville, KY, USA

Background: Chronic arsenic exposure through drinking water is a global health issue, affecting > 200 million people. Arsenic is a group I human carcinogen and clastogen causing chromosomal instability (CIN). Skin is the primary target organ for arsenic toxicity. miRNA dysregulation and CIN are suggested mechanisms of arsenic carcinogenesis. Preliminary data indicate that miR-186 is overexpressed in arsenic-induced squamous cell carcinoma relative to premalignant hyperkeratosis. Predicted targets of miR-186 are cell cycle regulators, thus overexpression of miR-186 potentially leads to CIN and aneuploidy, features of cancer. We showed that overexpressing miR-186 in HaCaT cells increased numerical and structural chromosomal abnormalities. Hypothesis: miR-186 overexpression drives malignant transformation of HaCaT cells by induction of CIN and potentially accelerates arsenic-induced transformation. Methods: Stable clones of HaCaT transfected with pEP-miR-186 expression vector or empty vector were maintained under puromycin selection. Selected clones exposed to 0 or 100 nM $NaAsO_2$ were cultured for 29 weeks. Anchorage independent growth was tested in soft agar colony formation assay. Giemsa banding was used to produce karyotypes and cytogenetic analysis for chromosomal aberrations was performed. Results: HaCaT overexpressing miR-186 and exposed to $NaAsO_2$ showed increased growth ability in agar in 12 weeks in contrast to vector control cells exposed to $NaAsO_2$. This group of cells also showed additional material of chromosome 1 and 20 in all cells analyzed and significant number of losses of chromosomes 13, 14, 15 and 16. The levels of E-cadherin were also reduced in all arsenite-exposed cells in 29 weeks compared to 12 weeks. Discussion: These results suggest that miR-186 overexpression exacerbates the arsenite-induced CIN and potentially is associated with accelerated skin carcinogenesis. The authenticity of the cells was validated by the presence of reported marker chromosomes. Only one clone and one timepoint were analyzed for each group and the presence of these karyotypic changes might be a feature of this specific clone and not an effect of arsenic toxicity. Thus, future research using additional clones is warranted.

Investigating the role of Paraoxonase 2 in non-small cell lung carcinoma proliferation

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Introduction
Paraoxonase 2 (PON2) is an intracellular enzyme with arylesterase and lactonase activity with near-ubiquitous tissue expression. Early research in PON2 biology established its role in protecting against atherosclerosis. More recent studies have implicated a role for PON2 in tumor survival and the development of drug resistance. Our work has demonstrated that PON2 is upregulated in tumors from patients with non-small cell lung carcinoma (NSCLC) compared to normal lung tissue, as well as in Ras-transformed human bronchial epithelial cells. Based on these observations, we hypothesized that PON2 may contribute to the survival and proliferation of NSCLC.
Objective
To test this hypothesis, we examined PON2's influence in proliferation and metabolism in PON2-knockout (PON2-KO) mice, tissue lysates, primary cells, and NSCLC cell lines using traditional molecular biology approaches, liquid chromatography-mass spectrometry (LC-MS), and high-resolution nuclear magnetic resonance (NMR).
Methods
PON2-KO C57BL/6 mice were generated using CRISPR/Cas9; PON2 status was confirmed by sequencing, western blot analysis, and enzymatic assay. Tracheal epithelial cells (TECs) from wild type (WT) or PON2-KO animals were analyzed for PON2 activity using LC-MS. Proliferation of control- or PON2-shRNA human bronchial epithelial cells (HBE) and human embryonic kidney (HEK-293T) cells was monitored for 96h. CRISPR/Cas9 was used to generate PON2-KO and vector control NCI-H1299 cells; intra/extracellular metabolites were quantified using NMR following 72h culture in uniformly-13C-labeled glucose. Statistical analysis was performed using Student's unpaired t-test.
Results
PON2 status does not affect mouse body or organ weight. Sequencing, western blot, and enzymatic assay confirm loss of PON2 expression in PON2-KO mice. Loss of PON2 expression does not alter proliferation of HBE or HEK-293T cells, but NCI-H1299 cells lacking PON2 expression exhibit decreased proliferation and reduced oxidative metabolism.
Conclusions
Together, these findings indicate PON2 is dispensable for mouse development and normal cell proliferation, but required for NSCLC proliferation and oxidative metabolism. Thus, PON2 may serve as a selective therapeutic target against NSCLC.

Sex Differences in Pulmonary Eicosanoid Metabolism in Response to Ozone Exposure

Michael Yaeger[1,2], Sky W. Reece[2], Brita J. Kilburg-Basnyat[2], Myles Hodge[2], Christine Psaltis[2], Bin Luo[2], Michael Armstrong[3], Nichole Reisdorph[3], Espen E. Spangenburg[4], Johanna L. Hannan[4], Robert M. Tighe[5], Saame Raza Shaikh[6], Kymberly M. Gowdy[1,2]

[1]Ohio State University, Columbus, OH 43210; [2] Department of Pharmacology and Toxicology, ECU, Greenville, NC; [3]School of Pharmacy, UC Denver, Denver, CO; [4]Department of Physiology, ECU, Greenville, NC; [5]Department of Medicine, Duke University, Durham, NC; [6]Department of Nutrition, UNC at Chapel Hill, NC.

Ozone (O3) is a criteria air pollutant known to increase the morbidity and mortality of cardiopulmonary diseases. Cardiopulmonary diseases are induced, in part, by a pulmonary inflammatory response characterized by recruited pulmonary immune cells, increased pro-inflammatory cytokines, and altered production of pro-inflammatory and pro-resolving lipid mediators. Recent evidence has demonstrated there are sex-dependent differences in the O3-induced pulmonary inflammatory response. However, if this dimorphic response is driven by pulmonary lipid mediators has yet to be examined. Therefore, we hypothesized there are sex-dependent differences in lipid mediator production following O3 inhalation. Male and female C57BL/6J mice were exposed to 1 ppm O3 for 3 hours and were necropsied 6 and 24 hours following exposure. Lung lavage was collected for cell differential and protein analysis, and lung tissue was collected for RNA analysis, lipid mediator and fatty acid quantitation, and immunohistochemistry. Comparable to other studies, O3 exposed female mice had increased airspace neutrophilia, and Cxcl1 and Cxcl2 expression. Additionally, females had significant increases in several pro-inflammatory lipid mediators including 12S-HETE, PGF2α, and PGE2 following O3 exposure. Specialized pro-resolving mediators (SPMs) 14(S)-HDHA, 17(S)-HDHA, PDX, and RvD5 were also significantly increased in female lung tissue when compared to males post exposure. Likewise, precursor fatty acids arachidonic acid (AA) and DHA were increased in female lung tissue at baseline and following O3 exposure. Curiously, these findings were independent of ovary producing hormones, as evidenced by examining ovariectomized female mice. Taken together these data indicate that O3 drives an augmented pulmonary inflammatory response in females that coincides with increased fatty acids in lung tissue, pro-inflammatory lipid mediators, and SPMs.

Zinc supplementation rescues cadmium-exacerbated, high fat diet-induced NADLD

Jamie L. Young[1], Lu Cai[1,2]

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Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver diseases effecting more than 25% of the world's population. Although obesity is a major risk factor for NAFLD, it does not account for all cases, suggesting the contribution of other factors such as environmental exposures. Exposure to the non-essential metal cadmium (Cd) is implicated in the development of NAFLD; however, the ability of early-life, in utero Cd exposure to influence the development of diet-induced NAFLD is poorly understood. Furthermore, studies do not take into account that such environmental exposures may be life-long and multigenerational. Therefore, we developed an in vivo two-hit model to study the effect of whole life, low dose Cd exposure and high fat diet (HFD) on NALFD. Additionally, we investigated the impact of dietary zinc supplementation on disease outcome as both obesity and Cd disrupt zinc homeostasis and zinc deficiency is common in both obese and NALFD patients. Adult male and female C57BL/6J mice fed normal diets (ND) were exposed to 0 or 5 ppm Cd-containing drinking water for 14 weeks before breeding. At weaning, offspring (F1) were fed ND or HFD containing 30 or 90 mg zinc/4057 kcal, representing normal and supplemented zinc diet, respectively, and continuously exposed to the same drinking water regiment as their parents. Water consumption and body weights were recorded weekly. DEXA scan technology was used to assess changes in body fat composition and intraperitoneal glucose tolerance tests were performed. Mice were sacrificed 24 weeks post-weaning. In addition to increasing HFD-associated body weight gain and insulin resistance, exposure to Cd exacerbated HFD-induced liver disease as indicated by plasma transaminases, liver to tibia ratios, histology, and hepatic triglycerides. Furthermore, HFD blunted the response of metallothionein, a major Cd detoxification protein in mice exposed to Cd suggesting a possible mechanism by which Cd enhances HFD-induced NAFLD. Zinc supplementation was able to rescue this phenotype reducing body weight gain, insulin resistance and protecting the liver injury and lipid deposition, possibly by induction of metallothionein. Overall, results from this study will provide insight into the mechanisms by which whole life, low dose Cd exposure enhances HFD-induced NAFLD and discern a potential therapeutic role for zinc.

Post-Doctoral Platform Presentations

2:00 - 3:00 pm

PRESENTATION TITLES, NAME & ABSTRACTS

Post-Doctoral

Electronic Cigarette Solvents, Pulmonary Irritants and Endothelial Dysfunction: Role of Acetaldehyde and Formaldehyde

Lexiao Jin, Jordan Lynch, Andre Richardson, Daniel J. Conklin

American Heart Association-Tobacco Regulation and Addiction Center, University of Louisville; Christina Lee Brown Envirome Institute, University of Louisville

After a decade of electronic cigarette (E-cig) use in the U.S., 3 elements persist: 1) E-cigs use propylene glycol (PG) and vegetable glycerin (VG) as solvents; 2) E-cigs continue to evolve; and, 3) uncertainty about E-cig use and cardiopulmonary disease risk. As all E-cigs use PG:VG, mice were exposed by inhalation to either PG:VG-derived aerosol or filtered air. As E-cig aerosols contain high levels of saturated aldehydes, mice were similarly exposed by inhalation to either formaldehyde (FA) or acetaldehyde (AA). Biomarkers of exposure and cardiopulmonary injury were monitored by mass spectrometry (urine metabolites); radiotelemetry (respiratory reflexes); isometric myography (aorta); and, flow cytometry (blood markers). Acute PG:VG exposure significantly affected multiple biomarkers including pulmonary irritant reflex (decreased respiratory rate, -50%); endothelium-dependent relaxation (-61.8±4.2%); decreased WBC (-47±7%); and, increased RBC (+6±1%) and hemoglobin (+4±1%) vs air control group. Other biomarkers were unchanged by PG:VG including aortic contractility and levels of circulating angiogenic cells (CACs) and platelet-leukocyte cell aggregates (PLAs). Notably, FA exposure recapitulated effects of PG:VG aerosol on pulmonary irritant reflex and endothelial dysfunction, whereas AA exposure did not induce either effect. To link PG:VG biomarkers of injury with FA or AA exposure, urinary formate and acetate were measured by GC-MS/MS after 6h exposure. Exposure to PG:VG aerosol significantly increased excreted acetate but not formate within the first 3h post-exposure. Neither FA nor AA exposure altered excretion of their primary metabolite, formate or acetate, respectively, compared with air-exposed controls. These data suggest that E-cig use may increase cardiopulmonary disease risk independent of the presence of nicotine and/or flavor constituents. Furthermore, perhaps FA, an abundant thermal degradation product of PG:VG, contributes to pulmonary irritation and endothelial dysfunction in E-cig users. This study indicates that FA levels in tobacco product-derived aerosols should be regulated to levels that do not induce biomarkers of cardiopulmonary harm.

Post-Doctoral

Vitamin D Modulates Corticosteroid Sensitivity in Allergic Airway Inflammation

Brandon W. Lewis[1], Devine Jackson[1], Josh Walum[1], Rodney D. Britt Jr[1,2]

[1]Center for Perinatal Research, Abigail Wexner Research Institute at Nationwide Children's Hospital, Columbus, OH, [2]Department of Pediatrics, The Ohio State University, Columbus, OH

Introduction: Airway hyperresponsiveness (AHR) and remodeling are hallmark features in severe pediatric asthma that persist even with corticosteroid treatments. Vitamin D deficiency in children has been associated with increased risk of developing asthma. In the present study, we examined the combined effects of calcitriol, a Vitamin D receptor VDR agonist, and fluticasone propionate (FP), a corticosteroid, on airway hyperresponsiveness in steroid-resistant allergic airway inflammation.

Methods and Results: To establish a mouse model of severe allergic airway inflammation, newborn mice were exposed to PBS or mixed allergen (MA; Aspergillus fumigatus, House Dust Mite, Alternaria alternata, and ovalbumin) in combination with a bacterial second-messenger, c-di-GMP, for 7 weeks. Mice were also administered intranasally vehicle or 50 ng/g calcitriol, active metabolite of Vitamin D, and injected intraperitoneally with vehicle, 1 mg/kg, or 3 mg/kg FP. Airway hyperresponsiveness (AHR) was assessed by performing methacholine challenge using the Scireq flexivent and bronchoalveolar lavage (BAL) was harvested for inflammatory cell infiltration analyses. AHR remained increased in c-di-GMP + MA mice treated with1 mg/kg or 3 mg/kg FP. However, treatment with 50 ng/g calcitriol alone and in combination with 1 mg/kg and 3 mg/kg FP reduced AHR in c-di-GMP + MA challenged mice. Mice challenged with c-di-GMP exhibited increased immune cell infiltration. Treatment with 3 mg/kg FP, but not 1 mg/kg FP, significantly reduced BAL immune cell infiltration. Treatment with calcitriol in combination with 1 mg/kg FP significantly reduced total BAL immune cell numbers. This reduction was contributed to by a decrease in total BAL eosinophils.

Conclusions: These data suggest that vitamin D may modulate corticosteroid sensitivity in allergic airway inflammation. Enhancement of corticosteroid sensitivity by vitamin D may involve reducing airway smooth muscle hypercontractility and remodeling. Activation of vitamin D receptor pathways may provide a therapeutic approach to improve corticosteroid sensitivity and alleviate asthma in patients with severe pediatric asthma.

These studies are supported by NIH R00 HL131682-05 (Britt).

Post-Doctoral

Acetylation of arylamine N-acetyltransferase 1 in breast cancer as a regulator of catalytic activity and expression

Raúl A. Salazar-González, Mark A. Doll, David W. Hein

Department of Pharmacology & Toxicology and James Graham Brown Cancer Center, University of Louisville School of Medicine, Louisville, Kentucky, USA

N-acetyltransferase 1 (NAT1) is a drug metabolizing enzyme that influences cancer cell proliferation and survival, especially in breast cancer. The mechanism for these effects is yet to be determined. Acetylation is an important Post-Translational Modification in the regulation of diverse cellular processes. Histone deacetylases (HDAC) and Sirtuins (SIRT), as protein deacetylases, may have an important role on the NAT1 acetylation status, affecting its catalytic capacity and having an impact on the downstream functions of this protein. The aim of the present work is to investigate the acetylation status of NAT1 in human breast cancer cells. Breast cancer cell lines MDA-MB-231 (ER-, PR-, HER2-) and ZR-75-1 (ER+, PR+, HER2+) were cultured in the presence of HDAC inhibitor Vorinostat (SAHA) or Sirtuin inhibitor Sirtinol. Under these conditions, NAT1 protein, gene expression as well as enzymatic activity were quantified. Acetylation of NAT1 protein was evaluated following an immunoprecipitation and acetyl-lysine quantification. Finally, Sirt1 and Sirt2 genes were silenced using siRNA and NAT1 protein, gene expression and catalytic activity were quantified. Data was analyzed using oneway ANOVA with Tukey post-hoc test, $p< 0.05$ was considered significant. The treatment of MDA-MB-231 or ZR-75-1 cells with increasing concentrations of SAHA resulted in 2 to 6-fold increase in NAT1 message expression ($p<0.01$); similarly, the NAT1 protein expression increased 2 to 3-fold ($p<0.05$) in both cell lines. Finally, the catalytic activity of NAT1 in the presence of SAHA increased 2-fold ($p<0.05$). Conversely, the chemical inhibition of Sirtuin activity produced decreases in the message, protein and catalytic activity, however, these changes were not statistically significant ($p>0.05$). Acetylated lysine in NAT1 was increased in the HDAC-inhibited cells ($p<0.01$) and Sirtuin-inhibited cells ($p<0.05$). Finally, silencing of Sirt1 and Sirt2 genes with siRNA resulted in reduced NAT1 protein expression ($p<0.01$) and NAT1 catalytic activity ($p<0.01$) but not NAT1 mRNA expression ($p>0.05$). These results provide evidence that chemical inhibition of HDAC and SIRT enhanced lysine acetylation of NAT1. HDAC but not SIRT inhibition enhanced NAT1 expression and activity in both MDA-MB-231 and ZR-75-1 breast cancer cells.

List of Other Abstracts Submitted/Accepted
(Abstracts on following pages)

PhD

Saeed Alqahtani
Shan Huang
Silvia Karim
Christine Kim
Li Xia
Idoia Meaza
Micaela Reeves
Ola Wasel

Post-Doctoral

Ana P. Ferragut Cardoso
James T.F. Wise

Undergraduate

Anusha Kotapalli
Angela Kyntchev

PhD

Exacerbated Acute Inflammatory Response by Nanoparticle Exposures in a Metabolic Syndrome Mouse Model

Saeed Alqahtani, Li Xia, Jonathan Shannahan

School of Health Sciences, Purdue University, West Lafayette IN

Nanoparticles are increasingly utilized in many applications, such as manufacturing processes, electronics, and consumer products, enhancing the potential for human exposures. Epidemiological assessments have established that individuals suffering from underlying diseases such as metabolic syndrome are increasingly sensitive to exposures; however, the mechanisms remain unknown. Our previous study demonstrated metabolic syndrome causes enhanced inflammation following nanoparticle exposure compared to healthy due to reductions in lipid mediators of inflammatory resolution. Our current study hypothesizes that specific resolution mediators are impaired in metabolic syndrome exacerbating inflammatory responses following exposure. To evaluate this hypothesis, healthy and metabolic syndrome were injected with saline (control) or specific precursors of resolution mediators 14-HDHA, 17-HDHA, or 18-HEPE at 1μg. After supplementation, mice were exposed to saline or 50μg of 20nm silver nanoparticles via oropharyngeal aspiration and necropsied 24 hours post-exposure. Again, metabolic syndrome enhanced pulmonary neutrophilic influx, inflammatory gene expression, and bronchoalveolar lavage inflammatory cytokine levels. None of the treatments altered the acute inflammatory response induced in healthy mice in response to silver nanoparticles. Treatment with 14-HDHA and 17-HDHA were determined to reduce exacerbated inflammatory responses in the metabolic syndrome mouse model to levels observed in the exposed healthy model. Additionally, targeted mass spectrometry measured altered pulmonary lipids while Western blots examined metabolic syndrome associated changes in resolution receptors that may impair resolution signaling. This data further demonstrates that dysregulation of resolution contributes to increased inflammation associated with metabolic syndrome. Specifically, decreases in specialized pro-resolving mediators of maresin-1 and resolvin D series produced by 14-HDHA and 17-HDHA appear to mediate exacerbations. A thorough understanding of mechanisms of inflammation is necessary for the development of treatment strategies and interventions.

PhD

Metallothionin rescues diabetic cardiomyopathy independent of Akt2 probably via activating ERK pathway

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To efficiently and safely prevent diabetic cardiomyopathy (DCM), a major cause of diabetic patient mortality, we have explored and confirmed that zinc supplementation could prevent DCM via inducing cardiac metallothionein (MT) in type 1 and type 2 mouse models. Then we further showed that MT prevents DCM via not only its anti-oxidative stress action, but also increasing Akt2 phosphorylation and associated glucose metabolic pathways. Here, mice with either global Akt2 knockout (Akt-KO), cardiomyocyte-specific overexpressing MT gene (MT-TG) or both (Akt2-KO/MT-TG) were used to define whether MT prevention of DCM is fully dependent on the role of Akt2-mediated glucose metabolism. Results showed that Akt2 is an important for the glucose metabolism since Akt2-KO mice exhibit a type 2 diabetes phenotype and develop diabetic cardiomyopathy (DCM, cardiac remodeling and dysfunction) along with reduced expression of several molecules that involve in regulating glucose metabolic pathway; however, unexpectedly Akt2-KO-associated DCM was almost fully prevented by overexpressed cardiac MT in the Akt2-KO/MT-TG mice. In addition, the cardiac protection by MT in Akt2-KO mice was accompanied by significantly preserving glucose metabolism pathway, such glycogen synthesis, and glycolysis. These results suggest that MT regulates the glucose metabolism pathways not only by regulating Akt2, but also by other pathways that can be vicariously regulated downstream in the absence of Akt2. We further found increased phosphorylation of ERK in the heart of Akt2-KO/MT-TG mice compared to those of Akt2-KO mice. This suggests that the protection against Akt2 deletion by MT high expression may be associated with the ERK pathway.

PhD

Exposure to Bisphenols A and its Analogues Activates Estrogenic Response and Alters Metabolic Endpoints in Zebrafish

Silvia Karim[1], Caroline Pinto[2], Ruixin Hao[2], Marina Grimaldi[3], Patrick Balaguer[3], Maria Bondesson[1]

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[2]Center for Nuclear Receptors and Cell Signaling, Department of Biology and Biochemistry, University of Houston, Houston, Texas 77204, USA.
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Background: Bisphenol A (BPA) is a plasticizer commonly used in lining of food cans, dye for printing receipts and it used to be common in baby bottles. Because of its widespread use, it is a common environmental pollutant. BPA acts as an estrogen and as such may affect embryonic development as well as adult health, and exposure to BPA correlates to metabolic disorders in humans. BPA analogues have been synthesized to be considered as replacement molecules for BPA. These analogues need to be thoroughly evaluated for their estrogenic activity and capacity to interfere with normal metabolism.
Objectives: Here we combined zebrafish-based assays to examine estrogenic activities of BPA and five of its analogues, BPAF, BPE, BPC, BPC-CL and BPS along with endogenous estrogen, 17-βEstradiol, *in vivo* and *in vitro*. We further investigated the effect of bisphenol exposure on the expression of metabolic genes involved in gluconeogenesis to examine the possible link between estrogenic activity and metabolic regulation.
Methods: We used *Tg(5×ERE:GFP)* and *Tg(pck1:Venus)* to study agonistic estrogenic effects of bisphenols *in vivo.* One to three days old zebrafish embryos were exposed to a concentration range of BPA, BPAF, BPE, BPC, BPC-Cl, BPS and 17β-estradiol, followed by imaging at day three to four by fluorescence microscopy. The fluorescence in images were quantified by ImageJ. The *in vivo* response was compared to *in vitro* effects of bisphenol exposure using reporter cells that express the zebrafish estrogen receptors driving expression of an ERE-luciferase reporter. qPCR was used to analyze expression of metabolic genes *in vivo*.
Results: Exposures to BPA and its analogs induced GFP expression in estrogen reporter fish. In reporter cell lines, whereas BPA and most analogs preferentially activated estrogen receptor alpha, BPC-Cl activated estrogen receptor beta. Exposures to some of the analogs further altered the expression of Pck1, the rate limiting enzyme in gluconeogenesis.
Conclusion: Transgenic estrogen reporter fish in combination with estrogen reporter cells efficiently can be used to assess estrogenic capacity of environmental pollutants. The estrogenicity of bisphenols promote emphasizing these chemicals for further analysis in higher vertebrates, and risk assessment for humans.

PhD

Assessing the mechanism of chronic arsenic-induced cell migration

Christine Kim, Joseph Chen, and Brian P. Ceresa

Department of Pharmacology and Toxicology, University of Louisville

Lung cancer is the leading cause of cancer death in the U.S. Although smoking is associated with lung cancer development, 20% of patients who die from lung cancer in the U.S., have never smoked, suggesting there are other risk factors. One of these risk factors is arsenic exposure. Fifteen percent of the U.S. population drinks domestic well water, and in many areas, this unregulated well water contains levels of arsenic that exceeds the Environmental Protection Agencies (EPA) recommended levels. Though there is a strong association between arsenic exposure and lung cancer development, the clear mechanism is unknown. In our previous study, we observed increased EGFR protein expression levels and activity, increased cell migration, and an increase of both transcription and protein levels of TGFα. To understand the mechanism of chronic arsenic-induced increased cell migration, we used time-lapse microscopy to measure the cell speed and the lengths of cell protrusion. We observed a significant increase in the cell speed and the lengths of cell protrusion in response to chronic arsenic exposure. The increased cell speed was reversed with AG1478, an EGFR inhibitor, but the length of cell protrusion was not affected. This suggests an alternative pathway of which arsenic acts to contribute to cell protrusion. In this study, we conclude that chronic arsenic increases cell speed in an EGFR-dependent manner, but cell protrusions increase independent of EGFR signaling. Thus, arsenic-induced lung epithelial cell carcinogenesis likely reflects the perturbation of multiple signaling pathways.

PhD

Metabolic Syndrome Enhances Nanoparticle-Induced mTOR Signaling via Alterations in the Biocorona

Li Xia, Sherleen Adamson, Saeed Alqahtani, Lisa Kobos, Jonathan Shannahan

School of Health Sciences, Purdue University, West Lafayette IN

Nanoparticles (NPs) interact with biomolecules forming biocoronas (BC) in biological environments. The BC is known to impact biological responses affecting NP biomedical applications. Metabolic syndrome (MetS) is an increasingly prevalent condition within our society and results in the formation of unique BCs. In this study, we examined differential cell signaling that may occur in MetS by using the BC. Rodent cell lines (rat endothelial cells and mouse macrophages) and human relevant Fe_3O_4 NPs without or with BCs were utilized. NPs were incubated in water, 10% normal serum, or 10% MetS serum to form BCs. Endothelial cells were exposed to NPs for 3, 12, or 24 h and differential cell signaling was discovered using a RNA-Seq approach. Functional enrichment of the differentially expressed genes identified time-dependent alterations in the activation of a number of pathways including DNA damage repair, oxidative stress, inflammation, and mTOR signaling. Specific examination of mTOR signaling demonstrated BC-dependent alterations within the pathway consisting of enhanced phosphorylation of AKT as well as total and phosphorylated mTOR. Additionally, markers of apoptosis (cleavage of caspase-3 and cytochrome c levels) were elevated only in cells exposed to NPs with a MetS BC. mTOR signaling induces apoptosis via inhibition of autophagy. Macrophages were exposed to NPs without or with BCs and decreased gene expression of LC3, a marker of autophagy, was observed for only NPs with a MetS BC. Treatment with rapamycin, an inhibitor of mTOR, inhibited alterations in LC3 expression. To understand up-stream regulation of mTOR, we utilized pharmacological inhibitors to reduce NP interactions with cell surface scavenger receptors. CD36 was determined to be the primary facilitator of NP internalization and is increasingly expressed on cells due to MetS. Treatment of cells with a CD36-specific ligand was determined to enhance mTOR gene expression to a similar degree as exposure to NPs with a MetS BC. Lastly, a number of processes down-stream of mTOR (insulin sensitivity and lipogenesis) were determined to be specifically modified due to exposure to NPs with a MetS BC. Together these findings demonstrate that the formation of a BC within MetS may result in enhanced mTOR signaling due to increased scavenger receptor interactions leading to exacerbated responses and progression of MetS associated diseases.

PhD

Particulate Hexavalent Chromium Altered the Expression of miRNAs Involved in Carcinogenesis Pathways

Idoia Meaza[1], Rachel M. Speer[1], Jennifer H. Toyoda[1], Yuan Lu[2], Qian Xu[3], Ron Walter[2], Maiying Kong[3], John Pierce Wise, Sr.[1]

[1]Wise Laboratory of Environmental and Genetic Toxicology, University of Louisville. [2] Texas Tech University.[3]Department of Bioinformatics and Biostatistics, University of Louisville

Hexavalent chromium is a global health concern and a known human lung carcinogen, predominately targeting the lung. Although the mechanism of Cr(VI)-induced carcinogenesis is not fully understood, it is clear that Cr(VI) induces gene expression changes. However, the data available on how Cr(VI) alters gene expression is more limited. We focused on the ability of Cr(VI) to alter microRNA (miRNA) and conducted the first study evaluating global changes of miRNA expression after Cr(VI) exposure in human lung cells. WTHBF-6 cells were treated for varying time periods with particulate Cr(VI) and RNA was isolated. We performed RNA sequencing analysis to evaluate how particulate Cr(VI), the most potent form of Cr(VI), alters miRNA expression profiles after acute (24 h) and prolonged (72 and 120 h) exposure in lung cells. We identified 958 unique miRNAs expressed across all our samples. Particulate Cr(VI) altered miRNA profile with concentration and time. Specifically, the number of significantly downregulated miRNAs increased with concentration and time, and the significantly upregulated miRNAs largely increased after 24h exposure at the intermediate concentration. Pathway analysis showed miRNAs altered by particulate Cr(VI) were predicted to target pathways of Cr(VI) carcinogensis. Specifically, we found that more upregulated miRNAs were predicted to target Cr(VI) carcinogenic pathways than downregulated miRNAs. These data suggest Cr(VI)-altered miRNA may play a key role in Cr(VI)-induced carcinogenesis.

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PhD

Toxicological findings of a recombinant cholera toxin B subunit variant with therapeutic potential in ulcerative colitis

[1]Micaela Reeves, [1,2]Nobuyuki Matoba

[1]Department of Pharmacology and Toxicology, University of Louisville; [2]James Graham Brown Cancer Center, University of Louisville

Background: The cholera toxin B subunit (CTB) is the nontoxic and homopentameric component of the holotoxin. Upon binding to GM1 ganglioside on the surface of epithelial cells, CTB mediates entry and retrograde transport through the endomembrane system and disengages the catalytic A subunit in the endoplasmic reticulum (ER). EPICERTIN (EPT) is a recombinant variant of CTB with a non-native C-terminal extension harboring an ER-retention motif, KDEL. We have found that increased ER-retention time resulting from this modification allowed EPT to induce an unfolded protein response and TGF-β signaling in colon epithelial cells, triggering wound healing activity in preclinical colitis models. The unique epithelial repair activity of EPT hints at its therapeutic potential in ulcerative colitis.
Objective: We aim to develop data supporting a first-in-human clinical trial with an EPT enema indicated for ulcerative colitis. Here, we evaluated the efficacy and toxicity of intrarectal (IR) administration of EPT in preclinical rodent models.
Results: IR administration of EPT at 0.1 and 1 μM in mice (0.6 and 6.1 μg/animal) with acute dextran sodium sulfate (DSS)-induced colitis resulted in decreased disease activity index scores and increased body weight recovery, supporting a target therapeutic dose of ≤1 μM in clinical testing. A dose-escalation study was performed following a single IR exposure at 1, 2 and 5 μM (61.4, 122.8 and 307 μg/animal) in male and female Sprague Dawley rats. No drug-related adverse effects were observed for clinical observations, clinical pathology, and gross necropsy even at the highest dose tested. A pharmacokinetics study was performed in male and female mice dosed a 1 or 10 μM (6.1 and 61.4 μg/animal) IV bolus EPT. Plasma samples were collected up to 24 h postdose. EPT concentrations were highest at first collection and decreased steadily until unquantifiable by 4 h. The elimination phase half-life was 0.26 to 0.3 h. When healthy and DSS colitis mice (n = 72) were dosed 1 or 10 μM EPT IR, marginal amounts of EPT were found in only 4 plasma samples scattered across groups and time points, suggesting that systemic exposure after IR administration is negligible.
Conclusion: These data support further development of EPT as a potential therapeutic for ulcerative colitis.

PhD

Developmental Toxicity of Perfluoroalkyl Substances Using Zebrafish Model System

Ola Wasel, Hanna King, Kathryn Thompson, and Jennifer Freeman

School of Health Sciences, Purdue University, West Lafayette

Perfluoroalkyl substances (PFAS) are synethetic compounds that are used in food packaging products, firefighting materials, electronics, cookware, carpets, furniture, clothing, and many other applications. PFAS are composed of a fluorinated carbon chain. PFAS are persistent in environment and bioaccumulate in organisms. The concerns of PFAS toxicity led to voluntarily phasing out of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by their manufacturer. PFOA and PFOS are both composed of an 8 carbon chain (C8). Shorter chain chemicals (such as perfluorobutyrate (PFBA, C4) and perfluorobutane sulfonate (PFBS, C4)) and compounds with chemical modifications (such as GenX, C6) were used as a replacement to the long chain PFAS (>C7 for COO^- containing PFAS or >C6 for $SO3^-$ containing PFAS) in order to increase their degradation potential. In this study, we compared toxicity of five PFAS in order to assess the role of chain length, functional group and chemical structure in their toxicity. We compared the toxicity of PFOS, PFOA, PFBS, PFBA and GenX using zebrafish (*Danio rerio*). To determine LC50 of each chemical, zebrafish embryos were exposed to a range of concentrations of each chemical within 1-hour post fertilization (hpf) through 120 hpf. The toxicity of these compounds was assessed by monitoring the survivability every 24 hours through 120 hpf. 120hpf-LC50 were determined using GraphPad 8.0 software. In addition, behavioral analysis using a visual motor response test was performed. For behavioral analysis, we used concentrations of 0, 4, 40, 400, and 4000 part per billion (ppb). The exposure was terminated at 72 hpf and the test was done at 120 hpf. Results showed that the 120hpf-LC50s were 23 parts per million (ppm) for PFOS, 566.5 ppm for PFOA, 2470 ppm for PFBS, >10000 ppm for PFBA, and 8617 ppm for GenX. Toxicity ranking was PFOS> PFOA> PFBS >GenX > PFBA. Based on these results, we can conclude that toxicity increases with increasing the chain length. Also, presence of sulfonate group increased toxicity for PFAS of a given chain length. Behavioral analysis showed that embryonic exposure to PFOS, PFBS, PFBA or GenX induced changes in the locomotor activities in larvae, while PFOA didn't cause any changes. Future work will focus on identifying the mechanism behind the observed behavioral changes.

Post-Doctoral

Chronic Arsenic Exposure Induces Unique Alternative Splicing Landscapes at Each Stage of Cutaneous Squamous Cell Carcinoma Development

Ana P. Ferragut Cardoso[1], Mayukh Banerjee[1], Laila Al-Eryani[1], Mohammed Sayed[2], Juw W. Park[2,3] and J. Christopher States[1]

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Chronic arsenic exposure in drinking water is associated with an increased risk of developing cancerous and non-cancerous diseases. Pre-mRNAs are often subject to alternative splicing that either includes or excludes exons in the mature mRNA resulting in synthesis of functionally distinct protein isoforms. The imbalance in isoform species can result in pathogenic changes in critical signaling pathways. This work examines the putative role of differential alternative splicing in arsenic-induced skin carcinogenesis. Multiple cultures of immortalized human keratinocytes (HaCaT), four each with 0 or 100 nM $NaAsO_2$, were maintained for 28 weeks. RNA-Seq was performed in cells harvested at 7, 19 and 28 weeks with subsequent rMATS analysis. At least 600 significantly different alternative splicing events at each tested time point were observed, comprising all the five main types of alternative splicing and occurring both in the ORF and the UTR of genes. Based on functional relevance *ELK4*, *MINOS1, SHC1* and *XRRA1* were selected for validation of predicted alternative splicing events at 7 weeks by RT-PCR. Interestingly, some of these events did not correspond to any known annotated isoform. For all genes, densitometric analysis of RT-PCR data corroborated the RNA-Seq alternative splicing predictions. Protein expression validation of the selected alternative splicing events was challenging as very few isoform specific antibodies are available. These results suggest that differential alternative splicing events could in part be responsible for the changing proteomic landscape with time in arsenic-induced carcinogenesis and highlight the complex and dynamic role of alternative splicing in cancer progression.

Post-Doctoral

Impact of Metals on Aromatic Amine N-Acetyltransferase Metabolism, in Human Lung Cells

James T.F. Wise, Raul A. Salazar-González, Mark A. Doll, and David W. Hein

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Humans are exposed to carcinogenic compounds via environmental and occupational exposures (*e.g.* pollution, cigarette smoke, and dyes). Compounds of concern that may occur in mixtures together are aromatic amines (*e.g.* 2-aminofluorene [2-AF]) and heavy metals (arsenic, cadmium, hexavalent chromium [Cr(VI)] and nickel). Arylamine *N*-acetyltransferases 1 and 2 (NAT1/2) are key to the metabolism of aromatic amines and their genotoxicity. A previous report indicated cadmium reduces NAT1/2 activities. However, the implication of cadmium or other heavy metals on the metabolism of aromatic amines by NAT1/2 remains unknown and whether exposure to heavy metals alters the aromatic amines genotoxicity or cytotoxicity. This research seeks to assess whether heavy metals will alter the *N*-acetyltransferase metabolism of aromatic amines and their subsequent genotoxicity and cytotoxicity in immortalized human lung epithelial cells (BEP2D) expressing NAT1 and NAT2. We will measure individual and combined effects of heavy metals and aromatic amines on NAT protein (*e.g.* activity and expression) and genotoxicity and cytotoxicity. Our preliminary "in cell" western results show that exposure to Cd(II) decreased protein expression NAT1/2 in BEP2D cells We also observed Cd(II) reduced para-aminobenzoic acid (PABA) and sulfamethazine (SMZ) *N*-acetylation by NAT1 and NAT2 respectfully. Specifically, in BEP2D cells relative to the untreated control 0.5, and 1 uM Cd(II) NAT1 *N*-acetylation of PABA was 88 and 43% and NAT2 *N*-acetylation of SMZ was 86 and 47%. Additionally, using yeast extract expressing recombinant human NAT1 or NAT2, cadmium inhibited activity *N*-acetylation of 2-AF of NAT1 and NAT2. For 0.4 and 4 uM Cd(II) reduced activity NAT1 to 11 and 8% of control and NAT2 activity to 21 and 13% of control. We anticipate this observed reduction of NATs expressions and activity will alter the toxicity of an aromatic amine during co-exposures with a heavy metal and have implications for understanding the toxicity of co-exposures.

Undergraduate

Behavioral alterations of a lead and atrazine mixture exposure during early development in the zebrafish model system

Anusha Kotapalli, Janiel Ahkin Chin Tai, Keturah Kiper, and Jennifer L. Freeman

School of Health Sciences, Purdue University, West Lafayette, IN USA

Lead (Pb) and atrazine (ATZ) are hazardous environmental toxicants present in drinking water systems by exposure through pipes of plumbing systems in households built prior to 1986 and herbicide runoff from broadleaf treated weeds, respectively. Pb is a known heavy metal having adverse human health effects, which contributes to a continued concern for global health. ATZ, on the other hand, is a known endocrine disrupting chemical (EDC) which has the potential to alter biological pathways and is also a suspected carcinogen. We hypothesize that Pb and ATZ mixtures result in a greater than additive toxicity, causing increased adverse health outcomes than single chemical exposure. In this study, the sublethal concentrations of chemicals used were: 3 ppb (µg/L) ATZ, 30 ppb ATZ, 10 ppb Pb, and 100 ppb Pb. The toxicity in this study was evaluated by testing the behavior of the larvae from all treatment groups after 120 hours post fertilization (hpf) using the Noldus DanioVision. The cumulative locomotor data was analyzed with an analysis of variance (ANOVA) and post-hoc least significant difference test when appropriate (α=0.05). Behavior data with 100 ppb Pb mixtures supports that the 3 ppb ATZ/100 ppb Pb and the 30 ppb ATZ/100 ppb Pb mixtures showed hyperactivity in almost all behavior parameters compared to the control treatment (0 ppb; $p<0.05$). An additional analysis of the light and dark phases will be performed to further evaluate the trends of larval behavior. Additionally, analysis is also being completed with 10 ppb Pb mixtures for comparison.

Undergraduate

Motor function changes in three genotypes of mice exposed to benzo[a]pyrene during gestation and lactation

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Benzo[a]pyrene (BaP) is a pollutant and a known carcinogen. Exposure from BaP can come from various sources such as vehicle emissions, tobacco smoke, and grilled food. BaP exposure has been linked to delays in neurological development in both animals and humans. We used a mouse model to mimic the human genetic variation in genes related to BaP metabolism. Pregnant mice were dosed with 10mg/kg/day BaP in corn oil-soaked cereal or the corn oil vehicle from gestational day 10 to weaning at postnatal day 25 (P25). Once these mice have reached early adulthood (notes as P60) a battery of behavioral tests was conducted. We used Rotarod and a Pole Climbing test to assess motor function and coordination. There was a significant main effect of genotype in the Pole Climbing test with *Ahr^{b}Cyp1a2(-/-)* having shorter latencies to turn and to descend the pole ($P < 0.01$). In the Rotarod test, there was a significant main effect of genotype on Days 1 and 2 ($P < 0.05$) for both *Ahr^{b}Cyp1a2(-/-)* and *Ahr^{d}Cyp1a2(-/-)* knockout mice. This replicates are previous findings and suggests that CYP1A2 has an important role in cerebellar development and/or function. However, we did not find a significant effect of BaP on motor function in any group. We have tested five cohorts of animals thus far and will continue testing to reach at least n=16 litters per group.

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